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The Role of MMP-2 on Satellite Cells and Hypertrophy of the Mouse Plantaris Muscle After Functional Overload

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THE ROLE OF MMP-2 ON SATELLITE CELLS AND HYPERTROPHY OF THE MOUSE PLANTARIS MUSCLE AFTER FUNCTIONAL OVERLOAD

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Introduction

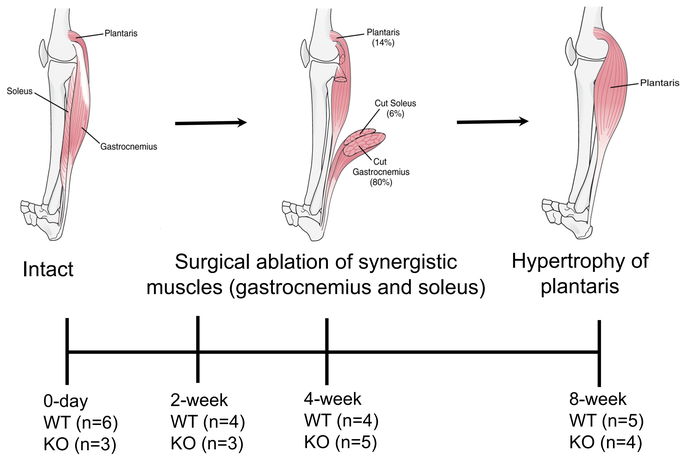
- Skeletal muscle is highly adaptive and displays remarkable regenerative capacity which allows it to increase in size during increased activity and atrophy with decreased activity.
- The extracellular matrix (ECM), which surrounds individual muscle fibers, is comprised of collagen fibers crucial for transmitting force and maintaining the functional integrity of fibers. (1)
- Satellite cells are resident stem cells found between the basal lamina and the cell membrane which synthesize components of the ECM and help with regeneration after injury contributing to the homeostasis of the muscle fiber. (5)
- Matrix metalloproteinases (MMPs) are zinc enzymes that are involved in the repair and regeneration of skeletal muscle. (2)
- MMP-2 breaks down type IV collagen leading to subsequent ECM remodeling and muscle hypertrophy following increased mechanical loading as occurs with exercise and functional overload (FO). (1)
- The purpose of the study was to determine the effects of MMP-2 on muscle hypertrophy after 0-, 2-, 4-, and 8-weeks FO in wild type (WT) and MMP-2 knock out (KO) mice.

Objectives

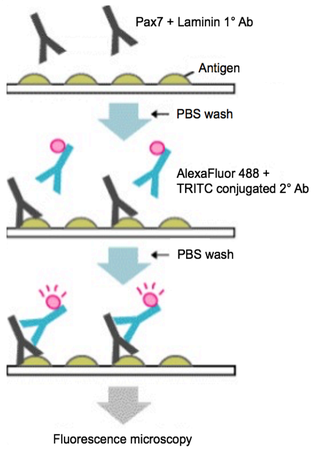
- Quantify and compare the number of satellite cells between WT and MMP-2 KO mice 0-day, 2-, 4-, and 8-weeks after FO.
- Quantify and compare the total number of fibers between WT and MMP-2 KO mice 0-day, 2-. 4-, and 8-weeks after FO.

Materials & Methods

Functional Overload



Immunohistochemistry



Serial cross sections of plantaris muscles were cut at 10 µm using a Leica CM 1950 cryostat and mounted onto slides.

Sections were fixed with 4% paraformaldehyde.

Sections were blocked with 5% goat serum and 0.4% Triton X-100 in PBS.

Sections were incubated with a primary antibody cocktail containing anti-laminin (1:500) and anti-Pax7 (1:20).

Sections were incubated with a secondary antibody cocktail containing AlexaFluor 488 conjugated goat anti-mouse (1:500) for Pax7 and TRITC conjugated goat anti-rabbit (1:500) for laminin.

Sections were mounted using an antifade mounting media containing DAPI to label nuclei.

Images of sections were taken using an epifluorescence microscope and satellite cells and muscle fibers were quantified using ImageJ.

Results

Body and Plantaris Muscle Weights

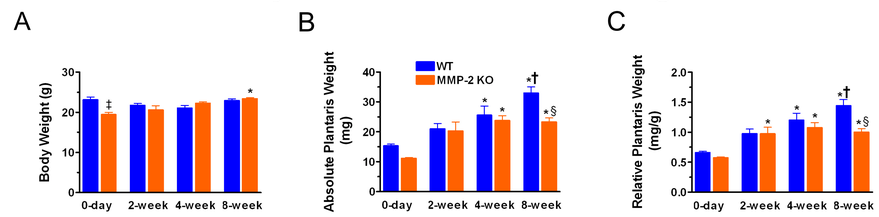


Figure 1. Body weight (A) for wild type (blue) and MMP-2 knockout (KO; orange) (n=4-6 at each time point) groups. Absolute (B) and relative to body weight (C) plantaris weights for each group. Values are means ± SEM. *, significantly different from their respective control; †, significantly different from 2-week WT; §, significantly different from 8-week WT at $p<0.05$.

Total Number of Muscle Fibers Quantified Using Laminin From Wild Type and MMP-2 Knockout Mice

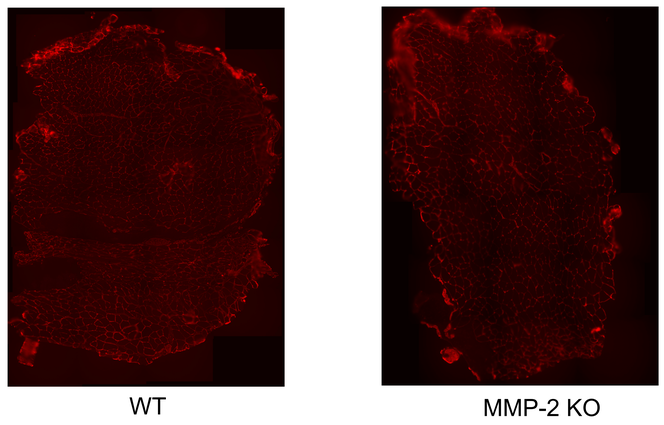


Figure 2. Plantaris muscles from the 8-week wild type (WT; left) and MMP-2 knockout (KO; right) mice were immunolabeled with anti-laminin to stain the basal lamina of individual muscle fibers.

Pax7 Immunofluorescence of Plantaris Muscle from Wild Type and MMP-2 Knockout Mice

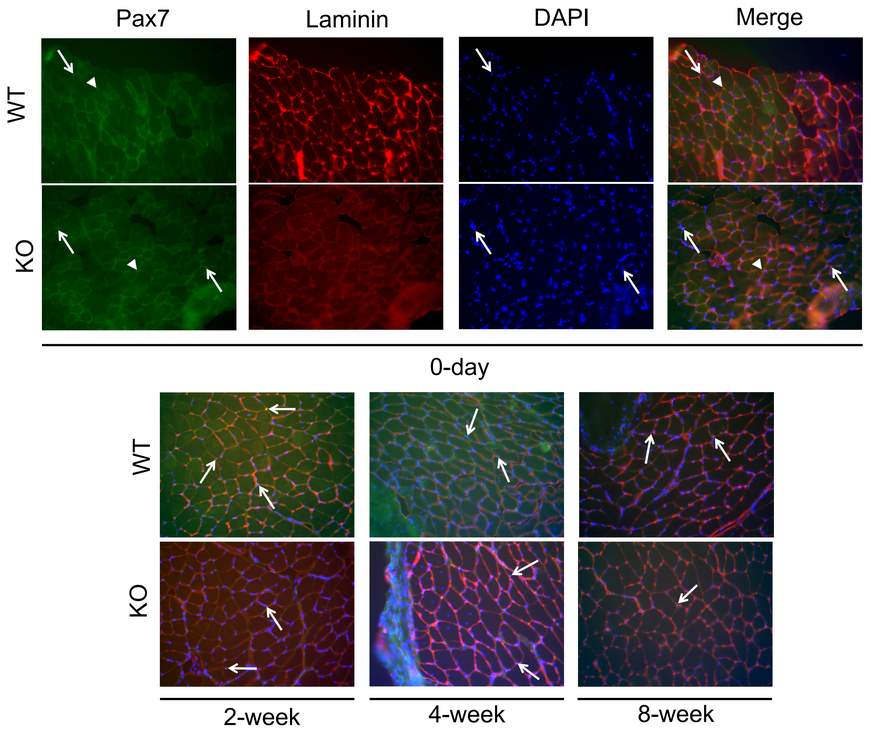


Figure 3. Plantaris muscles from 0-day, 2-, 4-, and 8-week wild type (WT) and MMP-2 knockout (KO) mice were immunolabeled with anti-Pax7 (green) for satellite cells, anti-laminin (red) for the basal lamina, and DAPI (blue) for nuclei. Arrows indicate nuclei that are Pax7+ and DAPI+. Arrowheads indicate nuclei that are not both Pax7+ and DAPI+.

Results

Total Muscle Fiber and Satellite Cell Quantification in Wild Type and MMP-2 Knockout Mice

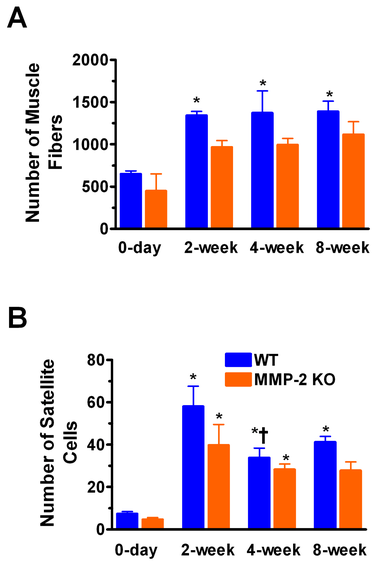


Figure 4. Total muscle fiber (A) and satellite cell (B) quantification from wild type (WT; blue) and MMP-2 knockout (KO; orange) plantaris muscles after 0-day (baseline), 2-, 4-, and 8-weeks FO. Values are means ± SEM. *, significantly different from respective control; †, significantly different from 2-week WT at $p<0.05$.

Conclusions

- The absolute plantaris muscle weights were significantly greater than 0-day after 4- and 8-weeks FO in both WT and MMP-2 KO mice, but not different at the 2-week timepoint.
- The relative plantaris muscle weights were significantly greater than 0-day after 2-weeks FO in the MMP-2 KO mice and after 4- and 8-weeks FO in both WT and MMP-2 KO mice
- There was a significant difference in the number of muscle fibers in the 2-, 4-, and 8-week WT mice compared to the 0-day mice showing that hyperplasia occurred, and this remained unchanged after 2-weeks.
- While there were less fibers in the MMP-2 KO mice, this was not statistically significant. There also were no differences in fiber number across all time points.
- There was a significant difference in the number of satellite cells between the 0-day and the 2-, 4-, and 8-week WT and MMP-2 KO mice. However, the MMP-2 KO mice had less satellite cells than the WT mice at all time points.
- There was a significantly higher number of satellite cells after 2-weeks FO compared to 4-weeks in both WT and MMP-2 KO mice.
- Future studies will focus on increasing the sample size for all time points in both the WT and MMP-2 KO groups.

References

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Acknowledgments

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